Eur J Oral Sci 2001; 109: 342–347
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European Journal of Oral Sciences
ISSN 0909-8836

A novel rat dentin mRNA coding only for dentin sialoprotein

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Ritchie HH, Li X. A novel rat dentin mRNA coding only for dentin sialoprotein. Eur J Oral Sci 2001; 109: 342–347. © Eur J Oral Sci, 2001

Dentin sialoprotein (DSP) is a major glycoprotein present in the mineralized dentin matrix that is expressed mainly by young and mature odontoblasts. Mutations in the DSP coding regions are linked to Dentinogenesis imperfecta I and II, indicating the importance of DSP in tooth formation. Previous studies have identified multiple mRNA transcripts in dentin that code for both DSP and phosphophoryns (PPs). Using reverse transcriptase-polymerase chain reaction (RT-PCR) to characterize these mRNA transcripts, we have identified a cDNA that codes for DSP, but not PP. This cDNA codes for a protein with 324 amino acids, 303 amino acids being identical to the published rat DSP sequence. However, the subsequent 21 amino acids are unique to this cDNA. Based on the coding sequence, the core protein is predicted to have a pI = 4.24, a net charge of −34, and to contain four potential N-glycosylation sites and six potential sites for phosphorylation by casein kinase. That the corresponding mRNA was present in day 5 molar tooth germs was confirmed using RNA protection assays. These data, therefore, identify a novel transcript in rat tooth germs that codes only for DSP (designated as DSPII).

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Key words: dentin sialoprotein; multiple transcripts; DSP isoform; dentin mineralization

Accepted for publication July 2001

Dentin sialoprotein (DSP) is a major glycoprotein that is present in the dentin matrix and whose expression is tightly associated with dentin formation (1, 2). It is rich in Asp, Ser, Glu, and Gly residues, contains 29.6% by weight of carbohydrates (including 9% sialic acid), and has a Mr of 53 kDa, as determined by sedimentation equilibrium analysis (3). A cDNA for DSP was identified from a rat incisor cDNA library (4) and later a coding sequence for mature rat phosphophoryn (PP), another major dentin non-collagenous protein, was identified at the 3′ end of the DSP sequence (5). The complete sequence analysis revealed a single transcript that coded for both mature DSP and PP proteins (5, 6). Similar findings were later obtained from mouse (7) and human tissues (8).

Mutations in the DSP coding regions are linked to Dentinogenesis imperfecta I and II, indicating the importance of DSP in tooth formation (9, 10). Furthermore, when rat DSP was used as a probe for *in situ* studies, DSP-PP transcripts were found to be expressed first in preameloblasts, then in young odontoblasts, and finally substantially expressed in mature odontoblasts actively synthesizing dentin (11). This transient expression profile may be indicative of sequential mineralization processes that are tightly regulated by the relative amounts or possibly types of DSP and PP present in the mineralizing dentin matrix.

Northern blot analyses with a DSP probe demonstrated that multiple transcripts around 4.6 kb and 1.5 kb were present in rat tooth germs (4, 12), and these transcripts were subsequently shown in rat (5, 13), mouse (7) and human (14) to contain a DSP-PP arrangement. Using reverse transcriptase-polymerase chain reaction (RT-PCR) to characterize these mRNA transcripts, we have identified a cDNA that codes for DSP, but not PP. That the corresponding mRNA was present in day 5 molar tooth germs was confirmed using RNA protection assays. We therefore present evidence for a novel transcript in rat tooth germs coding only for DSP, which has been designated DSPII.

Material and methods

RNA preparation and RT-PCR

Total RNA was extracted from day 5 rat tooth germs using RNAzol (Biotecx Laboratories, Houston, TX, USA). A cDNA pool was synthesized from total RNA using an oligo(dT) primer with an EcoRI adaptor (both obtained from Gibco, Gaithersburg, MD, USA) and reverse transcriptase (Boeringher Mannhiem, Indianapolis, IN, USA). Following standard procedures, this cDNA pool was denatured at 95°C for 4 min and amplified with a primer set comprising an oligoprimer corresponding to a rat DSP cDNA nucleotide sequence (4) at position 497 (5′aagaattcCTGGCCTTGCCAGTGACACGA3′) and an oligo(dT) primer. PCR was then performed as follows: denaturation, 1 min at 94°C; reannealing, 1 min at 45°C; and amplification, 2 min at 65°C for 30 cycles. This PCR product was analysed by Southern blot hybridization, and subsequently cloned into a pGEM7Zf(+) vector. Multiple PCR products, including a 500-bp fragment, were recognized by a ^{32}P-DSP probe. These multiple PCR fragments

were then digested with EcoRI and subcloned into a pGEM7Zf(+) vector. Several colonies were selected with a ^{32}P-DSP probe for further characterization. One of these colonies, containing a 500-bp insert, was sequenced and found to contain a partial DSPII coding sequence with a poly-A tail. A second PCR reaction to extend the 5′ sequence of this 500 bp fragment was performed using an oligo(dT) primer and a primer corresponding to a rat DSP cDNA sequence at position 13 (5′AGAGAATTCAATGAA-AACGAAGATA3′) (4), thus generating a PCR product comprising the complete DSPII coding sequence.

DNA sequencing

The PCR products recognized by a ^{32}P-labeled rat DSP probe were subcloned into a pGEM7Z(+) vector (Promega, Madison, WI, USA) using standard techniques. One of the cDNA clones contained a ~0.5 kb DNA insert and was used for DNA sequencing by universal primers. A PCR product containing the complete DSPII coding sequence was isolated and sequenced following standard procedures.

RNA protection assay

The subcloned DSPII cDNA was digested with Bgl I enzyme to generate a 1.7 kb DNA fragment. This 1.7-kb DNA fragment contained a unique portion of the DSPII cDNA sequence, a SP6 promoter and the partial pGEM7Zf(+) vector. A 322-nucleotide riboprobe was then generated using SP6 RNA polymerase and SP6 primer. This 322-nucleotide riboprobe (containing the unique 226-nucleotide DSPII fragment) was hybridized at 42°C to total RNA derived from rat day 5 tooth germs using an RNA protection kit (RPAIII; Ambion, Austin, TX, USA) and subsequently digested with RNAse T1 to remove the unpaired sequences. The resulting samples were then electrophoresed on 4% polyacrylamide gels and subjected to autoradiography (overnight exposure).

Results

Identification of DSPII cDNA

Using RT-PCR, we generated a cDNA pool from day 5 rat tooth germs and amplified the PCR fragments using an oligo(dT) primer with an EcoRI adaptor, and a primer corresponding to the published DSP coding sequence at nucleotide position 497 with an EcoRI adaptor (see Material and methods). Multiple PCR products, including a 500-bp fragment, were recognized by a ^{32}P-DSP probe (Fig. 1). These multiple PCR fragments were then digested with EcoRI and subcloned into pGEM7Zf(+). The resulting colonies were subjected to further selection with a ^{32}P-DSP probe. The DSPII clone, containing a 500-bp insert, was subsequently isolated and sequenced from one of these colonies (see Material and methods).

The sequence data obtained from the 500-bp DSPII clone showed identity (see Fig. 2) to that of previously reported DSP DNA (4) that was subsequently found to be part of the DSP-PP$_{240}$, DSP-PP$_{171}$, and DSP-PP$_{523}$ transcripts (5, 6, 15, 16). Interestingly, this 500-bp DSPII clone contained a poly(A) tail but it did not contain a PP coding sequence. This suggested the possibility that

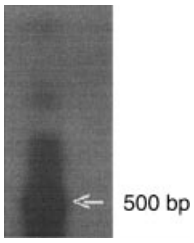

Fig. 1. Multiple RT-PCR products recognized by ^{32}P-DSP probe. PCR fragments from day 5 rat tooth germs were generated by 3′ RACE (see Material and methods). Southern blots were performed on these PCR products with a ^{32}P-DSP probe. A 500-bp band (as indicated by an arrow) and other bands were detected.

DSPII could be an alternative DSP transcript. To obtain a complete coding sequence for this DSPII transcript, a second RT-PCR was performed using an oligo(dT) primer and a 5′ primer containing the leader sequence (see material and methods). The resulting PCR fragment, with a size of ~1050 bp, was identified and subcloned into pGEM7Zf(+) for sequencing. The complete DSPII cDNA coding sequence and its deduced peptide sequence were obtained from this ~1050-bp fragment and are presented in Fig. 2.

Comparison of DSP-PP$_{171}$ and DSPII transcripts

Previously, we identified three DSP-PP transcripts: DSP-PP$_{240}$, DSP-PP$_{171}$ and DSP-PP$_{523}$ (5, 6, 15, 16). Each of these transcripts encodes a mature DSP protein as well as different PP isoforms containing 240, 171 and 523 amino acids, respectively. These three transcripts use different polyadenylation sites resulting in different 3′ noncoding sequences. As depicted in Fig. 3A, we found that the DSPII sequence shares sequence homology with that of DSP-PP$_{171}$ (15). For example, DSPII shares a 909-nucleotide sequence homology within the DSP coding region of the DSP-PP$_{171}$ transcript. This is also true for the DSP-PP$_{523}$ transcript (16) (not shown). Interestingly, the sequence between nucleotide positions 910 to 1050 in the DSPII transcript also shares sequence homologies with sequences located within the 3′ noncoding regions of the DSP-PP$_{171}$ (and DSP-PP$_{523}$) transcripts (Figs. 2, 3A).

Identification of DSPII transcripts in day 5 rat molar tooth germs

We next used an RNase protection assay to demonstrate whether the DSPII transcript was actually present in the day 5 molar tooth germ total RNA. We generated a riboprobe to a unique DSPII sequence, such that the presence of a DSPII transcript could be distinguished from that of other DSP transcripts and would be uniquely identified by a 226-nucleotide hybridization product (Fig. 3B). However, due to sequence identities in the 3′ poly-A regions, this probe was also expected to generate a 141 nucleotide hybridization product

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1        ATGAAAACGAAGATAATTATATATATTTGCATTTGGGCAACCGCATGGGCC
1          M  K  T  K  I  I  I  Y  I  C  I  W  A  T  A  W  A

52       ATTCCGGTCCCTCAGTTAGTGCCGCTGGAGAGAGACATTGTTGAAAAATCTGCCGACGTA
18         I  P  V  P  Q  L  V  P  L  E  R  D  I  V  E  K  S  A  D  V

112      CCCTTTCTAGCACATCCAGGAACCGCAGCACAGAATGAGTTGCATATCAACAACGCCACT
38         P  F  L  A  H  P  G  T  A  A  Q  N  E  L  H  I  N  N  A  T

172      ACGACGATTCCCCAAAGGGCAGTGAGCTAGGAAGGCAGGTACACTCTAATGGGGGTTAC
58         N  D  D  S  P  K  G  S  E  L  G  R  Q  V  H  S  N  G  G  Y

232      GAAAGAGACAGGAATGGCTCCGAGTCGATAGCCGTAGGAGGGAAGAGTTCTCCTACTCAG
78           E  R  D  R  N  G  S  E  S  I  A  V  G  G  K  S  S  P  T  Q

292      CCCATTTTAGCAAACGCACAGGGGAACAGTGCTAAAGAACGCGAGGACGTAGAAACATAC
98         P  I  L  A  N  A  Q  G  N  S  A  K  E  R  E  D  V  E  T  Y

352      GTCACGATGGGATACACGCAGGAGGAGAGAACAGCACAGCAAACGGCATCAGGGGCCAA
118        G  H  D  G  I  H  A  G  G  E  N  S  T  A  N  G  I  R  G  Q

412      GTAGGCATCGCTGAAAATGCCGAGGAAGCAAAGGAGAGTAAGGTACACGGGCAGCCTCAT
138        V  G  I  A  E  N  A  E  E  A  K  E  S  K  V  H  G  Q  P  H

472      AGGATACAAAAACTGGCCTTGCCAGTGACACGAGCCAGAATGGAGACGCCACCCTTGTC
158        Q  D  T  K  T  G  L  A  S  D  T  S  Q  N  G  D  A  T  L  V

532      AGGAAAATGAGCCTCAGGTAGCCGGAAGCAAGAATAGCACAAACCATGAGGTTGGAACA
178        Q  E  N  E  P  Q  V  A  G  S  K  N  S  T  N  H  E  V  G  T

592      CACGGGAGTGGAGTTGCTGCACAGGAAACGACGCCGCAGAGAGAAGGGGAGGGGAGTGAG
198        H  G  S  G  V  A  A  Q  E  T  T  P  Q  R  E  G  E  G  S  E

652      AACCAGGGGGCTGAGGTGACACCAAGCATTGGGGAAGGTGCTGGTTTGGATAATACTGAA
218        N  Q  G  A  E  V  T  P  S  I  G  E  G  A  G  L  D  N  T  E

712      GGAGTCCTAGCGGGAACGGGATAGAGGAGGATGAAGACACGGGTTCTGGTGATGGTGTG
238        G  S  P  S  G  N  G  I  E  E  D  E  D  T  G  S  G  D  G  V

772      GGTGCAGACGCAGGAGATGGAAGGGAGAGTCACGATGGCACGGAGGGCCACGAGGGCCAG
258        G  A  D  A  G  D  G  R  E  S  H  D  G  T  E  G  H  E  G  Q

832      TCTAGTGGGGGAAACAATGACAACAGAGGTCAGGGTTCAGTTAGTACTGAAGACGATGAC
278        S  S  G  G  N  N  D  N  R  G  Q  G  S  V  S  T  E  D  D  D

892      TCTAAAGAACAAGAAGGCTGCCCCCCATCACACCCACTCATCAAAGTTGTGATCACCAAA
298        S  K  E  Q  E  G  C  P  P  S  H  P  L  I  K  V  V  I  T  K

952      CCCTGGGTGGAAAACTCATTGTAAACTGTATGTGAAATCTAAAGGAAGAGATAATGAACT
318        P  W  V  E  N  S  L  *

1012     TCAGTATTATAATAAACATCTATTTATACAAAAAAAAAA 1050
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representing DSP-PP_{171} (Fig. 3B). These two protected sequences were subsequently identified and are shown in Fig. 4. An expected 85-nucleotide hybridization fragment, representing the protected DSP coding region of DSP-PP_{171} (Fig. 3B), was not detected (Fig. 4), which may have been due to the precipitation conditions used for this procedure.

Using the NIH Image J program for densitometric comparison, we determined the relative amounts of DSPII transcripts (i.e., the protected 226-bp nucleotide

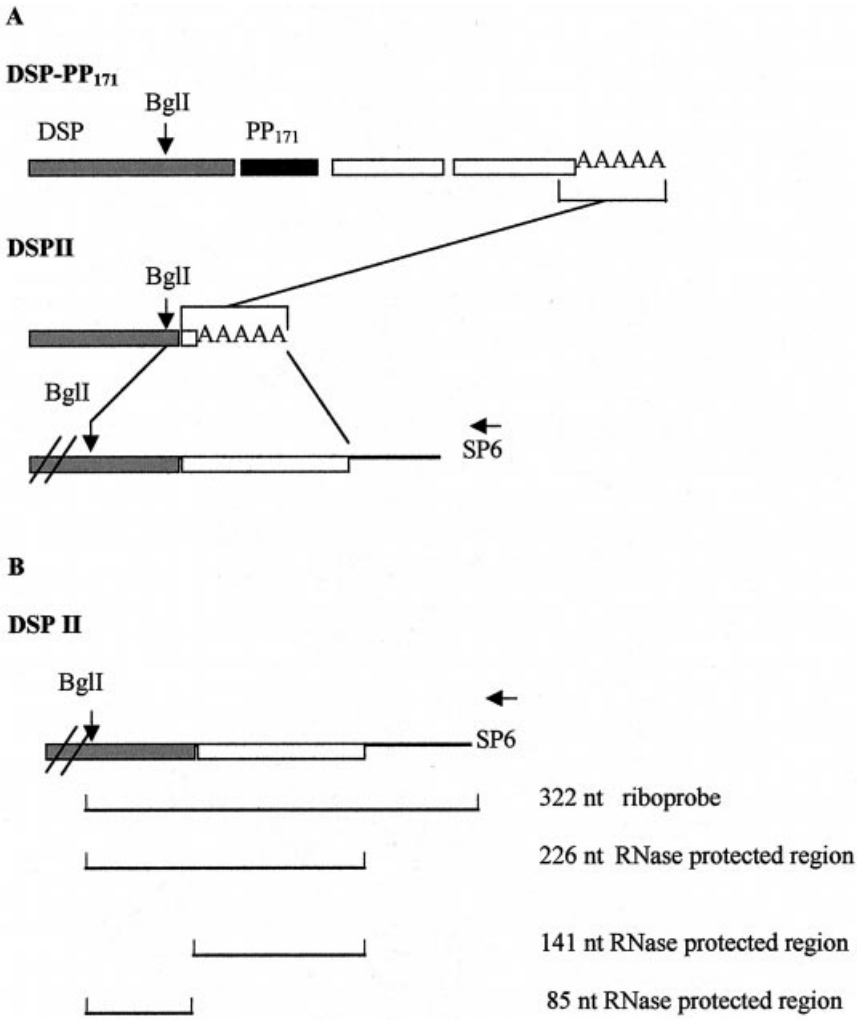

Fig. 3. DSPII and DSP-PP_{171} cDNA sequence homologies and riboprobe generation. A) Sequence homology between DSPII and DSP-PP_{171} cDNAs. DSP-PP_{171} is comprised of a DSP coding sequence (▬), a PP_{171} coding sequence (■) and a 3′ noncoding sequence (▭) with a poly (A) tail. DSPII is comprised of a DSP coding sequence (nucleotide positions 1–909; ▬) which shares sequence identity with DSP-PP_{171}. The Bgl I site (nucleotide position 825) is indicated by an arrow (↓). The DSPII sequence shares sequence homology (▭) with the 3′ noncoding sequence of DSP-PP_{171} (as shown by connected brackets). B) Riboprobe protection of a 226-nucleotide DSPII transcript. A 322-nucleotide riboprobe was generated from the SP6 promoter within a Bgl I DNA fragment. This 322-nucleotide riboprobe (bracketed) contains a 85 base DSP coding sequence, a 3′ (141 base) DSPII sequence, and a partial vector sequence. Following hybridization of the riboprobe with the day 5 rat tooth germ total RNA, a 226-nucleotide riboprobe sequence (bracketed) will be protected by DSPII transcripts.

Fig. 2. DSPII cDNA sequence and its deduced amino acid sequence. The translation start site, ATG, is located at position 1. The leader sequence comprises amino acids 1–17 (i.e., nucleotide positions 1–51). The putative secreted DSPII comprises 307 amino acids and shares a 286 amino acid identity (i.e., nucleotide positions 52–909) with the published rat DSP peptide sequence (4). The C-terminal 21 amino acid run (i.e., amino acid positions 304–324, nucleotide positions 910–972) is unique for DSPII. A 46-bp non-coding region is located 3′ to the stop codon at nucleotide position 973. The polyadenylation signal is located at nucleotide position 1022 (underlined). Four potential N-glycosylation sites (i.e., Asn-X-Ser) (17, 18) are underlined and in bold font. Six potential casein kinase I and II phosphorylation sites (D/E-X-X-S/T and S/T-X-X-D/E) (19–22) are underlined and shaded. D: Asp, E: Glu, X: any amino acid, S: Ser, T: Thr.

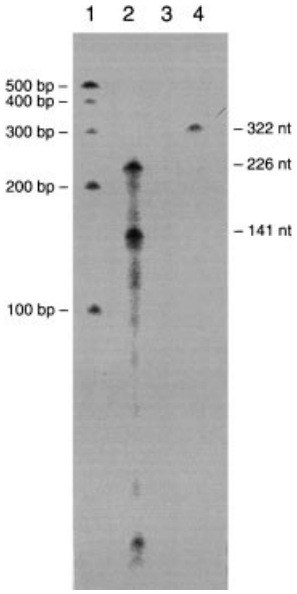

Fig. 4. The presence of DSPII transcripts in day 5 rat tooth germs. RNA protection assays were performed using a ^{32}P-DSPII riboprobe (see Fig. 3B and Material and methods) and day 5 rat tooth germ total RNA. Lane 1: RNA size markers. Lane 2: Day 5 tooth germ total RNA (10 μg) hybridized with the ^{32}P-DSPII riboprobe and treated with RNAse T1. The protected 226-nt region demonstrates the presence of DSPII RNA while the protected 141-nt region demonstrates the presence of DSP-PP$_{171}$ and DSP-PP$_{523}$ RNA. Lane 3: Control: Yeast tRNA (10 μg) was hybridized with the ^{32}P-DSPII riboprobe and treated with RNAse T1. Lane 4: ^{32}P-labeled DSPII Riboprobe (322 nt).

Table 1

Comparison of amino acid composition of the deduced rat DSPII, the published rat DSP protein and the native DSP protein

	Rat DSPII	Nat DSP[a]	Rat[b] DSP$_{406}$	Rat[b] DSP$_{421}$
pI	4.24	N/A	4.26	4.27
Net charge	−34	N/A	−43.6	−44.5
G+N	14	14	15	15
G	15	16	16	16
E+Q	17	18	17.5	17
S	9.5	11	11	12
A	7.5	7	6.7	6.4
H	4	4	3.7	3.8
I	4	4	3.45	3.6
K	4	3	4.2	4
L	4	3	3.2	3.1
P	6	4	4.4	4.3
R	3	3	2.7	2.9
T	6	7	6.9	6.7
V	6	4	3.9	3.8
Y	0.7	0.5	0.5	0.7
C	0.3	0.3	0.3	0.3
F	0.3	2.7	0.3	0.3
W	0.3	0	0	0

[a]The native rat DSP protein (2).
[b]The C-terminal cleavage sites at amino acid positions 406 and 421 generate two DSP proteins: DSP$_{406}$ and DSP$_{421}$ (25).

fragments) to that of DSP-PP transcripts (i.e., the protected 141-bp nucleotide fragments) in day 5 rat tooth germ total RNA. We found that the DSPII transcripts represented 40% of the DSP-related transcripts in this total RNA sample.

Characterization of the putative DSPII protein product

The DSPII cDNA contained a 972-nucleotide coding sequence capable of generating a 324 amino acid peptide including a 17-amino acid leader sequence. While the N-terminal 303 amino acids of the putative DSPII protein product are identical to those of the published native DSP amino acid sequence (4), it then diverges by having a unique C-terminal 21 amino acid run (Fig. 2). The calculated net charge of the mature DSPII before being glycosylated and phosphorylated would be −34 with a pI = 4.24 while that of DSP is −43 with a pI = 4.10 (Table 1). Four potential N-linked glycosylation sites (Asn-X-Ser/Thr) (17, 18) were detected at positions 55, 82, 128, and 189 (Fig. 1). Six potential casein kinase I/II phosphorylation sites (D/E-X-X-S/T and S/T-X-X-D/E) (19–22) also were found at amino acid positions 57, 226, 253, 278, 292 and 293 (Fig. 1). The amino acid compositions from the DSPII deduced peptide, the published deduced rat DSP peptide, and the native rat DSP are shown in Table 1. These composition comparisons show nearly identical values for all of the amino acids except for phenylalanine.

Discussion

Using RT-PCR, we have identified a novel DSP transcript, designated as DSPII, that only codes for DSP. This DSPII transcript encodes a 324-amino acid peptide, including a 17-amino acid leader sequence, and shares a 303-amino acid identity with that of the published rat DSP (4). However, the C-terminal 21 amino acids in DSPII diverge from those of the published rat DSP. The DNA sequence coding for this divergent C-terminal peptide was derived from the 3′ noncoding sequence of DSP-PP$_{171}$ (Fig. 3A) and DSP-PP$_{523}$ (not shown) transcripts. Taking advantage of this unique C-terminal sequence, we were able to make a ^{32}P-labelled riboprobe containing this unique sequence as well as the 3′ noncoding sequence of DSPII (see Fig. 3B). Using this riboprobe in an RNA protection assay, we have identified in day 5 rat tooth germ total RNA a protected 226-nucleotide hybridization product representing the DSPII transcript. This result indicates that DSPII mRNA is actually present in 5 day molar tooth germ total RNA, and excludes the possibility that the DSPII cDNA generated by the RT-PCR process was an artifact.

Ritchie and co-workers have demonstrated that multiple DSP-PP transcripts are present in rat tooth germ total RNA (4). Upon further cloning and sequencing, these transcripts were found to contain coding sequences for mature DSP and PP proteins in rat (5, 6, 13), mouse (7) and human (14) molar teeth. The DSP-PP arrangement was also found at the genomic level (6, 23) in rat and mouse genomic DNA. The rat genomic organization

showed that DSP-PP_{171}, DSP-PP_{240} and DSP-PP_{523} are likely generated from the DSP-PP gene (16). Given its shared sequence homologies with DSP-PP_{171} and DSP-PP_{523}, DSPII was likely derived from a DSP-PP transcript. This is the first report of a DSP transcript that does not contain a PP sequence.

The finding of DSPII cDNA is consistent with the detection of several DSP native protein isoforms in rat dentin (24). DSPII likely encodes the smaller DSP isoform reported by Butler (24). Additional experiments are needed to further examine how this novel DSPII cDNA transcript is generated, and how its temporal and spatial expression is related to PP expression during dentinogenesis. This work suggests that multiple regulatory and processing steps may facilitate dentin mineralization by generating multiple mature DSP and mature PP protein isoforms during the dentinogenesis process.

Acknowledgements – We want to express our appreciation to Drs. David G. Ritchie and Lee-Ho Wang for their helpful discussion and comments. This work was supported by NIH grant DE11442 (HHR).

References

1. Butler WT, Bhown M, DiMuzio MT, Linde A. Non-collagenous proteins of dentin. Isolation and partial characterization of rat dentin proteins and proteoglycans using a three-step preparative method. *Collagen Rel Res* 1981; **1**: 187–199.
2. Butler WT, Bhown M, D'Souza RN, Farach-Carson MC, Happonen RP, Schrohenloher RE, Seyer JM, Somerman MJ, Foster RA, Tomana M, van Dijk S. Isolation, characterization and immunolocalization of a 53-kDal Dentin Sialoprotein (DSP). *Matrix* 1992; **12**: 343–351.
3. Butler WT. Sialoproteins of bone and dentin. *J Biol Buccale* 1991; **19**: 83–89.
4. Ritchie HH, Hou H, Veis A, Butler WT. Cloning and sequence determination of rat dentin sialoprotein, a novel dentin protein. *J Biol Chem* 1994; **269**: 3698–3702.
5. Ritchie HH, Wang L-H. Sequence determination of an extremely acidic rat dentin phosphoprotein. *J Biol Chem* 1996; **271**: 21695–21698.
6. Ritchie HH, Ritchie D, Wang L-H. Six decades of dentinogenesis research. Historical and prospective views on phosphohoryn and dentin sialoprotein. *Eur J Oral Sci* 1998; **106**: 211–220.
7. MacDougall M, Simmons D, Luan X, Nydegger J, Feng J, Gu T. Dentin phosphoprotein and dentin sialoprotein are cleavage products expressed from a singly transcript coded by a gene on human chromosome 4. *J Biol Chem* 1997; **272**: 835–842.
8. Gu K, Chang S, Slaven M, Clarkson B, Rutherford R, Ritchie HH. Human dentin phosphophoryn nucleotide and amino acid sequence. *Eur J Oral Sci* 1998; **106**: 1043–1047.
9. Xiao S, Yu C, Chou X, Yuan W, Wang Y, Bu L, Fu G, Qian M, Yang J, Shi Y, Hu L, Han B, Wang Z, Huang W, Liu J, Che Z, Zhao G, Kong X. Dentinogenesis imperfecta 1 with or without progressive hearing loss associated with distinct mutations in DSPP. *Nat Genet* 2001; **27**: 201–204.
10. Zhang X, Zhao J, Li C, Gao S, Qiu C, Liu P, Wu G, Qiang B, Lo W, Shen Y. DSPP mutation in dentinogenesis imperfecta shields type II. *Nat Genet* 2001; **27**: 151–152.
11. Ritchie HH, Berry J, Somerman M, Hanks C, Bronckers ALJJ, Hotton D, Papagerakis P, Berdal A, Butler W. Dentin sialoprotein (DSP) transcripts: developmentally sustained expression in odontoblasts and transient expression in preameloblasts. *Eur J Oral Sci* 1997; **105**: 405–413.
12. Butler WT, Ritchie HH. The nature and functional significance of dentin extracellular matrix proteins. *Int Dev Biol* 1995; **39**: 169–179.
13. George A, Srinivasan R, Thotakura S, Veis A. The phosphophoryn gene family: identical domain structures at the carboxyl end. *Eur J Oral Sci* 1998; **106**: 221–226.
14. Gu K, Chang S, Ritchie HH, Clarkson B, Rutherford R. Molecular cloning of human dentin sialoprotein-phosphophoryn gene. *Eur J Oral Sci* 2000; **108**: 35–42.
15. Ritchie HH, Wang L. The presence of multiple DSP-PP transcripts. *Biochim Biophys Acta* 2000; **1493**: 27–32.
16. Ritchie H, Wang L-H, Knudtson K. A novel rat phosphophoryn isoform: Nucleotide sequence and genomic organization. *Biochim Biophys Acta* 2001; in press.
17. Marshall R. Glycoproteins. *Annu Rev Biochem* 1972; **41**: 673–702.
18. Bause E. Structural requirements of N-glycosylation of proteins. Studies with proline peptides as conformational probes. *Biochem J* 1983; **209**: 331–336.
19. Pinna L, Meggio F, Marin O, Perich J, Boldyreff B, Issinger O-G. Casein kinases: An atypical class of ubiquitous and pleiotropic protein kinases. *Adenine Nucleotide Cellular Energy Transfer and Signal Transduction* 1992; 269–279.
20. Flotow H, Roach P. Synergistic phosphorylation of rabbit muscle glycogen synthase by cyclic AMP-dependent protein kinase and casein kinase I. Implications for hormonal regulation of glycogen synthase. *J Biol Chem* 1989; **264**: 9126–9128.
21. Flotow H, Graves P, Wang A, Fiol C, Roeske R, Roach P. Phosphate groups as substrate determinants for casein kinase I action. *J Biol Chem* 1990; **265**: 14264–14269.
22. Flotow H, Roach P. Role of acidic residues as substrate determinants for casein kinase I. *J Biol Chem* 1991; **266**: 3724–3727.
23. Feng J, Luan X, Wallace J, Jing D, Oshima T, Kulkarni A, D'Souza R, Kozak C, Macdougall M. Genomic organization, chromosomal mapping and promoter analysis of the mouse dentin sialoprotein (DSPP) gene, which codes for both dentin sialoprotein and dentin phosphoprotein. *J Biol Chem* 1998; **273**: 9457–9464.
24. Butler WT. Dentin matrix proteins and dentinogenesis. *Connect Tissue Res* 1995; **33**: 59–65.
25. Qin C, Cook R, Orkiszewski R, Butler WT. Identification and characterization of the carboxyl-terminal region of rat dentin sialoprotein. *J Biol Chem* 2001; **276**: 904–909.